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COMMENTARY

The AhR–Nrf2 Pathway in Keratinocytes: On the Road to Chemoprevention?

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The ligand-activated transcription factor AhR mediates the cutaneous stress response toward a variety of environmental noxae and is therefore currently of interest for modern preventive medicine. In this issue, Tsuji *et al.* identify the antifungal agent ketoconazole as an inducer of AhR signaling and the Nrf2 antioxidant response in human keratinocytes. Ketoconazole-stimulated nuclear translocation of Nrf2 and its cytoprotective effects against oxidative stress strongly depend on a functional AhR. This newly identified AhR–Nrf2 pathway opens up new opportunities to prevent and treat inflammatory skin diseases.

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AhR and Nrf2 are key regulators of drug metabolism

The AhR, a ligand-dependent transcription factor, is an important regulator of drug metabolism that is activated by a broad spectrum of low-molecular-weight compounds, including dioxins, polycyclic aromatic hydrocarbons, plant polyphenols, and tryptophan photoproducts (Abel and Haarmann-Stemmann, 2010; Rannug and Fritsche, 2006). In the absence of a ligand, the AhR is trapped in a cytosolic multiprotein complex consisting of heat shock protein 90, tyrosine kinase c-src, and other cochaperones. Upon ligand binding, this complex dissociates and the AhR shuttles into the nucleus, dimerizes with ARNT, and binds to so-called xenobiotic-responsive elements in the promoter region of genes to stimulate their expression. Prototype AhR target genes encode for the phase I enzymes cytochrome P450 (CYP) 1A1, CYP1A2, and CYP1B1. These monooxygenases introduce functional groups into lipophilic chemicals to enhance their water solubility. The resulting metabolites are then conjugated to hydrophilic molecules, including activated sulfate, glutathione, and glucuronic acid. The expression of several enzymes responsible for these phase II reactions, such as various glutathione S-transferases and UDP-glucuronosyltransferases, are also regulated in an AhR–ARNT-dependent manner.

In addition, many of these phase II enzymes are transcriptionally coregulated by antioxidant-responsive elements (AREs) located adjacent to the xenobiotic-responsive elements (Haarmann-Stemmann and Abel, in press). These AREs are recognized by the transcription factor Nrf2, the master regulator of the cellular antioxidant response. In the cytoplasm Nrf2 is bound to kelch-like ECH-associated protein 1 (Keap1), which prohibits Nrf2 nuclear translocation and thus facilitates constant proteasomal degradation. Upon oxidative stress, dissociation of Nrf2 from Keap1 permits Nrf2 shuttling into the nucleus, where it binds to small Maf proteins to form a transcriptionally active complex to induce ARE-dependent responses (Haarmann-Stemmann and Abel, in press). Because exposure to AhR ligands such as dioxins, polycyclic aromatic hydrocarbons, and flavonoids results in the CYP-mediated formation of reactive oxygen species (ROS) and electrophilic metabolites, the interaction between AhR and Nrf2 signaling is probably a crucial enhancer of cytoprotection against toxic by-products of the AhR-dependent detoxification process.

New insight into the molecular mechanisms of AhR–Nrf2 cross-talk

Although earlier studies revealed that expression of Nrf2 can be modulated via the ligand-activated AhR, and vice versa (Miao *et al.*, 2005; Shin *et al.*, 2007), the work by Tsuji *et al.* (2012, this issue) considerably expands our view on the cross-talk between these intracellular sensor molecules. The investigators used normal human epidermal keratinocytes treated with ketoconazole (KCZ), a widely used imidazole antifungal agent that exhibits anti-inflammatory properties in addition to its therapeutic effects against *Malassezia* spp. and related skin pathogens. KCZ exposure stimulated nuclear translocation of the AhR, leading to enhanced expression of *CYP1A1*, an observation in line with previously reported AhR-activating properties of KCZ in human HepG2 hepatoma cells (Korashy *et al.*, 2007).

More interesting is the observation that KCZ treatment resulted in a nuclear translocation of Nrf2 without intracellular ROS production. Using transient RNA interference, the investigators further demonstrated that the KCZ-induced nuclear translocation of Nrf2 strongly depends on a functional AhR cascade, as illustrated in Figure 1. Surprisingly, the "classic" AhR ligand benzo(*a*)pyrene (BaP) was not capable of inducing Nrf2 nuclear translocation, pointing to a ligand-specific phenomenon. As mentioned above, the *Nrf2* gene locus contains functional binding sites for AhR–ARNT, which would explain an upregulation of Nrf2 expression but not an activation of processed Nrf2 protein. Therefore, another molecular link may contribute to the AhR-dependent nuclear accumulation of Nrf2.

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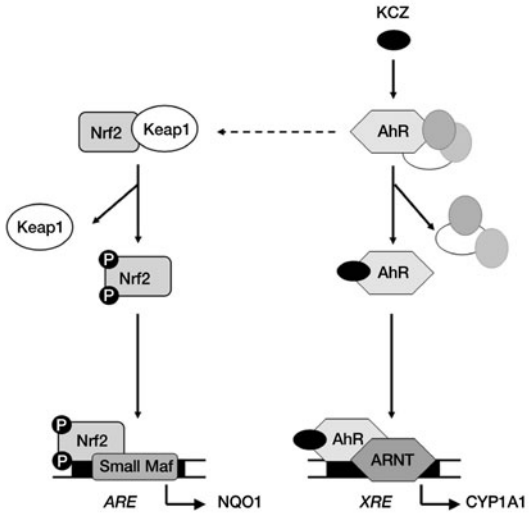

Figure 1. Activation of the AhR–Nrf2 pathway by the fungicide ketoconazole (KCZ). KCZ binds to the cytosolic AhR, resulting in its nuclear translocation and subsequent dimerization with ARNT. The AhR–ARNT complex binds to xenobiotic-responsive elements (XREs) in the promoters of target genes (e.g., *CYP1A1*) and stimulates transcription. Simultaneously, activation of AhR initiates dissociation of the Nrf2–kelch-like ECH-associated protein 1 (Keap1) complex via an unknown molecular mechanism, but one that probably includes activation of protein kinases. Phosphorylated Nrf2 translocates in the nucleus and binds to small Maf proteins. This transcriptionally active complex recognizes antioxidant-responsive elements (AREs) in promoter sequences and induces expression of target genes, e.g., NAD(P)H:quinone oxidoreductase 1 (NQO1).

Besides disturbance of the Nrf2–Keap1 complex through interaction of its cysteine residues with electrophiles, an important step in the dissociation of Nrf2–Keap1 is phosphorylation of the Nrf2 protein in the cytoplasm. This stabilization step is mediated by several kinases, including protein kinase C, phosphatidylinositol-3-kinase, and mitogen-activated protein kinases (MAPKs). Interestingly, ligand-dependent activation of the AhR is accompanied by stimulation of protein kinase C and activation of MAPK signaling (Haarmann-Stemmann *et al.*, 2009).

We have previously identified the AhR as a crucial mediator of the UVB stress response in human keratinocytes, which triggers activation of the epidermal growth factor receptor (EGFR) and downstream MAPK cascades upon UVB exposure. The initializing event for this pathway is the intracellular absorption of UVB radiation by tryptophan, resulting in the formation of the photoproduct 6-formylindolo[3,2-b] carbazole (FICZ) (Fritsche *et al.*, 2007). FICZ is a high-affinity ligand of the AhR that stimulates both induction of the CYP1A1 response through the classic AhR–ARNT pathway and activation of the soluble tyrosine kinase c-src. c-src is released in the cytoplasm upon ligand binding to AhR, and it can interact directly with cell surface receptors, such as the EGFR. This in turn activates downstream MAPK signaling cascades and finally results in the upregulation of target genes, e.g., *cyclooxygenase-2* (*COX*-2) (Fritsche *et al.*, 2007). Hence, it is tempting to speculate that KCZ activates AhR in keratinocytes, leading to the stimulation of MAPK signaling via the c-src–EGFR route. The activated MAPK may then phosphorylate Nrf2, a process that may contribute to, but not solely explain, KCZ-induced Nrf2 nuclear accumulation. Because BaP did not influence Nrf2 activation, unidentified ligand-specific effects of KCZ are probably involved.

AhR-mediated Nrf2 activation is cytoprotective

Tsuji and colleagues (2012) demonstrated the functional importance of the observed AhR–Nrf2 interaction by introducing different ROS producers into their cell system. Treatment of normal human epidermal keratinocytes with tumor necrosis factor-α led to intracellular ROS formation and enhanced IL-8 production, and both can be abolished by KCZ coexposure. Interestingly, the ROS-inhibiting property of KCZ is diminished in cells that lack either a functional AhR or Nrf2 system. In addition, KCZ exposure dose-dependently reduced BaP-induced ROS production and 8-hydroxydeoxyguanosine generation, as well as IL-8 secretion. Because CYP1A1 is the main BaP-metabolizing enzyme, one could speculate that the reduced production of ROS upon BaP exposure observed in the coexposure experiments is attributable to the simultaneous inhibition of CYP1A1 enzyme activity by KCZ. Indeed, it has been reported that KCZ, although widely used as specific inhibitor of CYP3A4, can decrease CYP1A1 enzyme activity in human intestinal tissues and human HepG2 hepatoma cells (Korashy *et al.*, 2007; Paine *et al.*, 1999). This would also explain the observation that KCZ alone did not produce intracellular ROS. Because Tsuji *et al.* (2012) were able to reverse the KCZ-mediated inhibition of all three end points by transient knockdown of Nrf2, the inhibition of CYP1A1 alone cannot explain this process. An enhanced expression of certain Nrf2-dependent BaP-detoxifying enzymes (e.g., NAD(P)H:quinone oxidoreductase 1) may contribute to the reduced toxicity of BaP in KCZ-treated keratinocytes. In aggregate, these findings emphasize the relevance of Nrf2 for the toxicity of BaP and related AhR ligands, independent of CYP1A1-mediated catalysis.

Chemoprevention: activation or inhibition of AhR?

The findings by Tsuji *et al.* (2012) revealed an anti-inflammatory role of AhR in the skin that is attributable to the activation of the cytoprotective Nrf2 system. This is a new and unexpected function because we have shown that activation of AhR by UVB irradiation leads to the induction of (ROS-producing) CYP1A1 and pro-inflammatory COX-2 enzymes (Fritsche *et al.*, 2007). On the basis of these data, we and others proposed that the UVB-induced activation of the AhR signaling cascade is

Clinical Implications

- The anti-inflammatory property of ketoconazole may be attributable to its capability to interfere with AhR and Nrf2 signaling.
- The AhR-dependent activation of the Nrf2 pathway opens new opportunities in the development of treatment modalities for inflammatory skin diseases.

detrimental to skin by contributing to photocarcinogenesis and photoaging (Agostinis *et al.*, 2007; Tigges *et al.*, 2011). In line with this concept, it was reported that activation of the AhR by its prototype ligand 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) led to enhanced COX-2 expression in rodents (Vogel *et al.*, 2000) and increased IL-8 secretion in U937 macrophages (Vogel *et al.*, 2007), again pointing to a pro-inflammatory function of the AhR. In addition, transgenic mice expressing a constitutively active form of the AhR in keratinocytes develop inflammatory skin lesions that resemble typical atopic dermatitis (Tauchi *et al.*, 2005). Finally, most recent studies from our group indicate that the AhR in keratinocytes serves as a negative regulator of nucleotide excision repair, again emphasizing that AhR inhibition may be beneficial for human skin (Haarmann-Stemmann *et al.*, 2011).

The apparent discrepancies between these reports and the current report by Tsuji *et al.* (2012) may best be reconciled by the existence of ligand-specific effects. Indeed, Tsuji *et al.* observed a relatively slight induction of the CYP1A1 response upon KCZ treatment, and the affinity of KCZ to the AhR ligand-binding domain is probably not comparable to that of high-affinity ligands such as TCDD or FICZ. We therefore postulate that KCZ is a so-called "partial agonist" of the AhR: a weak activator of AhR in sole treatment but a potent antagonist in coexposure with ligands of higher affinity. This phenomenon was previously described for several AhR antagonists of plant origin, for example, luteolin, curcumin, and indigo (Abel and Haarmann-Stemmann, 2010). Regarding the use of such molecules in chemopreventive approaches, we recently proposed that compounds that both attenuate ligand-activated AhR signaling and stimulate the Nrf2 system are probably the most potent candidates to prevent the severe health effects provoked by environmental noxae (Haarmann-Stemmann and Abel, in press). The inhibition of activated AhR may reduce CYP1-catalyzed metabolic activation of chemicals and the Nrf2-initiated antioxidant defense may simultaneously scavenge the remaining reactive metabolites. Whether the antifungal agent KCZ is one such promising substance has yet to be determined. Another interesting task will be to verify whether other AhR agonists (such as endogenously generated FICZ or food-contained polyphenols) also activate Nrf2 and its downstream enzymes in an AhR-dependent manner. It should be noted that, in addition to keratinocytes, melanocytes and epidermal Langerhans cells express functionally active AhR and thus may also be KCZ targets (Jux *et al.*, 2009, 2011).

The newly discovered connection of the AhR and the Nrf2 pathways directly links drug metabolism and cellular antioxidant response, thereby opening new opportunities to develop and design novel strategies that prevent and treat inflammatory skin diseases.

CONFLICT OF INTEREST

The authors state no conflict of interest.

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